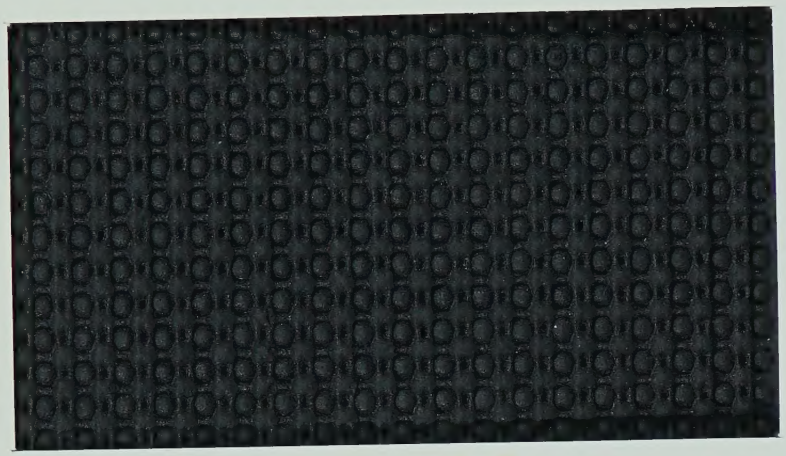
Umeå University

Some aspects of physiological processes of lichens and pine trees, affected by air pollutants. Considerations of measurements of SO_2 effects on photosynthesis.

Jan-Erik Hällgren

Postal address
S-901 87 UMEÅ
Sweden
Telephone
090/12 56 00



Some aspects of physiological processes of lichens and pine trees, affected by air pollutants. Considerations of measurements of SO_2 effects on photosynthesis.

Jan-Erik Hällgren

AKADEMISK AVHANDLING

som med tillstånd av rektorsämbetet vid Umeå universitet för erhållande av filosofie doktorsexamen, framlägges till offentlig granskning tisdagen den 14 februari kl. 10.00 vid Botanik-Fysiologi Hufo, seminarierum B.

This thesis is based on the following papers, which will be referred to in the text by the given Roman numerals.

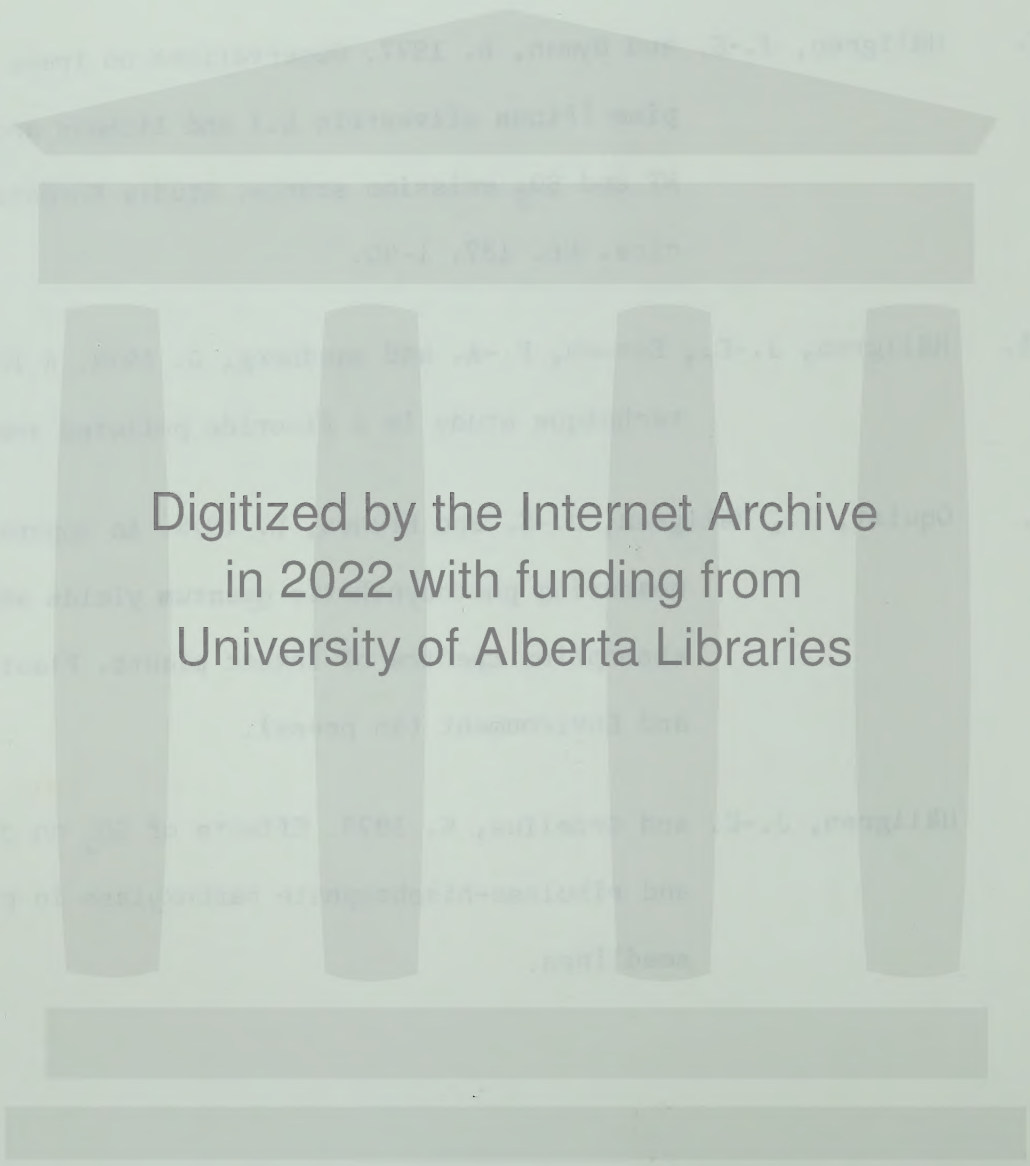
I. Hällgren, J.-E. and Huss, K. 1975. Effects of SO_2 on Photosynthesis and Nitrogen fixation. Physiol. Plant. 34: 171-176.

II. Hällgren, J.-E. and Nyman, B. 1977. Observations on trees of Scots pine (Pinus silvestris L.) and lichens around a HF and SO_2 emission source. Studia Forestalia Suecica. Nr. 137. 1-40.

III. Hällgren, J.-E., Esseen, P.-A. and Sandberg, G. 1978. A lichen and technique study in a fluoride polluted area.

IV. Öquist, G., Hällgren, J.-E. and Brunes, L. 1978. An apparatus for measuring photosynthetic quantum yields and quanta absorption spectra of intact plants. Plant, Cell and Environment (in press).

V. Hällgren, J.-E. and Gezelius, K. 1978. Effects of SO_2 on photosynthesis and ribulose-bisphosphate carboxylase in pine tree seedlings.



INTRODUCTION

Studies of the effects of sulphur dioxide (SO_2) on plants were first initiated in the late nineteenth century. Because of its intrinsic interest to both chemists and biologists the existing literature is now voluminous, and has frequently been reviewed [1-28].

Except in proximity to emission sources, air pollutant concentrations are some six orders of magnitude lower than the most abundant natural components of the atmosphere. The background level of sulphur dioxide (SO_2) in the atmosphere is 1-4 ppb.[29] In urban areas with a considerable variation in SO_2 concentration the long time mean values are considered to be about ten times the background level.

With regard to this, most studies of the effects of air pollutants on plants have been criticised because they have tended to concentrate on visible symptoms of damage in experiments where unrealistically high concentrations have been used. Furthermore, most experimental fumigations involve a single pollutant although this is rarely the prevailing situation in urban and industrial environments. Usually the experimental studies have been carried out with steady levels of pollutants. Again, this is far from the normal situation. It has been claimed that any study that uses high levels of pollutants, however short-term, is likely to be stigmatised as unrealistic despite the fact that transients which

---

Concentrations are often quoted in ppb, pphm, ppm.[29]

These are converted to $\mu g\ m^{-3}$ as follows.

ppb $\times\ 10^{-3}$ $\times$ molecular weight (MW) $\times$ 40,9 = $\mu g\ m^{-3}$ (at normal atm pressure)

quantity in $\mu g\ m^{-3} \times \frac{1}{MW \times 10^6} \times 0{,}0244 \times 10^8$ = pphm

undoubtedly occur in urban and industrial environments may even exceed these levels. While there might be justification for criticism of this kind, experimental and field studies of a more rigorous nature have led to important advances in our knowledge of the way air pollutants attack metabolic processes, or affect the plant's functioning at the physiological level. There is also experimental evidence that the effects of SO_2 on photosynthesis are accentuated in combination with other pollutants.[30-31] SO_2 has a somewhat special position among air pollutants since sulphur is one of the essential plant nutrients. As a consequence, both harmful and beneficial effects are conceivable.

Plants sometimes show specific responses to pollutants and therefore may be used as biological indicators[32] for determination and evaluation of effects of pollutants. In the field, some lichens seem to be more sensitive to atmospheric pollution by SO_2 than other plants.[22] Thus, the absence of lichens from urban / individual industrial areas or even whole countries[33] has been associated with atmospheric pollution by SO_2. It has been suggested that sensitive species might be affected already at 10-30 /ug SO_2 m^{-3} air.[22,24] In comparison it is estimated that sensitive coniferous plants such as Pinus silvestris (L.) are adversely affected at mean concentrations of SO_2 over 80 /ug m^{-3} during the vegetation season.[12] Some individuals and races or cultivars of species[34-41] can vary greatly in their sensitivity to SO_2. Variation in sensitivity can also be observed, within the plant, in leaves of different ages.[7,28,42,43]

The inhibition of photosynthesis is often regarded as the first sign of SO_2 action on plants.[17] However, in some species physiological processes such as nitrogen fixation or other processes mediated by enzymes sensitive to SO_2[21] may be equally or even more rapidly inhibited (I).

Dealing with environmental pollution promises to be one of the many urgent problems in the years to come. It can be foreseen that as the subject develops it will be increasingly necessary for biologists to consider the interaction between air pollutants and plants in natural ecosystems, in agriculture and in forestry.

DISCUSSION OF THE EFFECTS OF SULPHUR DIOXIDE IN PHOTOSYNTHESIS AND NITROGEN FIXATION

An initial stimulation of photosynthesis at low SO_2 concentrations or even by sulphuric acid treatment[44], has been observed in studies with chloroplasts[45,46], algae[47], lichens[48] and higher plants[30]. Generally this effect is short-lived and followed by a substantial inhibition with prolonged fumigation time[30].

It has generally been difficult to explain the observed SO_2-effects on photosynthesis and, to elucidate the underlying mechanism(s). Most studies of SO_2 action on photosynthesis have been related to the photosynthetic performance of the plant e.g. expressed as the net CO_2 exchange under more or less well defined conditions. From these studies, however, it has not been possible to obtain information about the underlying mechanism(s). Therefore, an alternative approach has been to study the reactions of SO_2 *in vitro* in an attempt to assess the relevance of such reactions to physiological conditions *in vivo*. Although only a selection of the numerous physiological or biochemical processes in a plant may be monitored in any one experiment, these studies are needed to give an understanding of SO_2 action on photosynthesis and to identify sensitive mechansims.

There exist several plausible explanations for the SO_2 action(s) on photosynthesis. The reported effects of SO_2 and other gaseous pollutants on stomatal activity and behaviour are of importance. An extensive review of this topic has recently been made by Mansfield.[49] Several investigators have stressed the acidifying effects of SO_2 being caused by formation of either sulphurous acid or after oxidation, sulphuric acid.[50,51] The conversion of chlorophyll to phaeophytin has been one concomitant site of the action of SO_2 on photosynthesis.[e.g. 24]

The sulphur is normally required in a reduced state in the plant, which is obtained after reduction of sulphate (SO_4^{2-}). Whether exogenous sulphite (SO_3^{2-}) fits into the scheme for SO_4^{2-} reduction is doubtful.[52] However, the ability to oxidize SO_3^{2-} to SO_4^{2-} is considered to be correlated with resistance to toxicity.[53]

Recent studies have indicated that the predominant site for SO_3^{2-} oxidation is in the chloroplast.[45,46,54] In isolated chloroplasts the oxidation is shown to be dependent on the photosynthetic electron transport and probably initiated through the univalent reduction of oxygen.[45,54,55] It has also been suggested that low SO_3^{2-} concentrations could stimulate the electron transport and enhance the CO_2 fixation.[46] The SO_4^{2-} formed by oxidation will be reduced to the sulphide (S^{2-}) level via the assimilatory pathway of sulphate reduction. The chloroplast has also been shown to be the site for SO_4^{2-} reduction, and this process is connected with the electron transport in photosynthesis which delivers the energy for SO_4^{2-} activation.[52,56-58] Chloroplast preparations of plant leaves contain sulphite reductases and are able to reduce SO_3^{2-} to S^{2-}[59] (protonated to H_2S).[60,61,62,63] This reaction is also dependent on the electron flow of photosynthesis.

Whether toxic amounts of HSO_3^- or SO_3^{2-} will be formed inside chloroplasts during fumigation with SO_2 has been disputed.[64] Bisulphite compounds formed

by SO_2 fumigation have been shown to exert nonspecific effects on the chloroplast membrane and also to disturb fluxes of ions[47,65-67] and to cause leakage of photosynthetic products.[47] Ultrastructural investigations on chloroplast membranes[68-73] have shown that the first symptoms are swelling of grana thylakoids and fret channels between the grana and granulation of the stroma. It is of interest that the effect is reversible after short time fumigation with SO_2.[68] One of the most important aspects of protein and membrane disruption is the cleavage of disulphide linkages by SO_3^{2-}.[74] Since the structure and function of several proteins are highly dependent on the integrity of the disulphide bounds, the breakage of these should gradually inactivate several enzymes and alter membrane proteins. It has also been shown recently that the lipid composition of chloroplast membranes can be altered by SO_2.[75]

Although it has been claimed that the sulphur anions have no direct inhibitory effect on photosynthetic electron transport[45,76-78], a number of investigations show that the oxygen evolution of intact chloroplasts is inhibited by 'SO_2', HSO_2^-, SO_3^{2-} and SO_4^{2-}.[73, 79-81] It is concluded by Baldry et al.[80] that SO_4^{2-} does not interfere with the mechanisms of O_2 evolution, or electron transport, but that by affecting photophosphorylation, or ATP utilization it indirectly inhibits the conversion of 3-phosphoglycerate to 1,3-diphosphoglycerate, and hence the final stages of hydrogen transfer. That SO_3^{2-} and SO_4^{2-} are capable of inhibiting phosphorylation has convincingly been demonstrated in several different systems tested.[82-88] Jagendorf[89] has recently proposed an hypothesis for the SO_4^{2-} inhibition. However, there is some doubt that inhibition of photophosphorylation could entirely explain the observed inhibition of photosynthetic oxygen evolution by SO_2.[79] It was furthermore shown that the inhibition of oxygen evolution was relieved by addition of superoxide dismutase (SOD), indicating the participation of the superoxide radical.[81]

Several other possible targets for SO_2 in the photosynthetic process have been suggested. As a nucleophilic agent the sulphite sulphur, even in low concentrations,[90,91] interacts directly with the pyridinium moiety of e.g. NAD and NADP. HSO_3^- will form a reversible adduct with NAD in analogy to its reaction with other nucleophiles.[92] SO_3^{2-} combines with NADP to give a hydroxypyridine-4-sulphonic acid which is capable of forming an undissociable complex with dehydrogenase and thereby inhibits the hydrogen transfer. SO_3^{2-}, is a strong ligand and binds to iron-heme containing enzyme centres.[93] This has been used to explain SO_2 blockage of metallo-enzyme mediated processes in photosynthesis. SO_3^{2-} is also known to bind to vitamins.[48,92,94] A wellknown reaction is thiamine cleavage.[95,96,97] The reactivity of SO_3^{2-} is such[98] that one might expect reactions with phenolic compounds and quinones, and with compounds such as aldehydes, especially α-β-unsaturated aldehydes and ketons. The reaction with aldehydes and ketones is applicable to 5 and 6 carbon sugars formed in the dark reaction of photosynthesis. The formation of addition compounds,[99,100] such as α-hydroxysulfonates, which are inhibitors of enzyme catalyzed reactions is questioned.[18,19]

The effect of SO_2 on the enzymes involved in the dark reaction of photosynthesis has gained considerable interest.[21,101-108] It has been generally suggested that the central point of attack could be represented by the competitive inhibition of the enzyme RuBP-carboxylase with respect to CO_2.[101,106,10] (What is the internal concentration of "SO_2" in the chloroplast?). Other enzymes such as phosphoenolpuryvate carboxylase[102] and malic enzyme(s)[103,104] are also inhibited. SO_3^{2-} acts on NAD- and NADP-dependent malate dehydrogenase in several ways. The most important finding so far is the reported effect on light regulation of these and other enzymes.[109,110] The capa-

city for light regulation has been shown to be strongly reduced after treatment of the particulate fraction from broken chloroplasts with only 10 /mol SO_3^{2-} in the light and in the dark. From these results it seems possible that SO_3^{2-} concentrations in the order of magnitude generated <u>in vivo</u> could disrupt metabolic regulation in both higher and lower plants.[110] SO_3^{2-} causes alteration of carbon metabolism, and the expenditure of energy and matter for detoxication is important in relation to plant growth and development.

The effects of SO_2 on nitrogen fixation have not been investigated at the biochemical level. Hence direct effects on the enzyme are not known. Several of the explanations given for the effects of SO_2 on photosynthesis certainly could be applicable to nitrogen fixation as well.

NOTES ON THE EFFECT ON CHLOROPHYLL

Very often the effect of SO_2 on photosynthesis has been explained by chlorophyll breakdown or degradation. For a recent review of lichen studies the reader is referred to Le Blanc and Rao.[24] The phaeophytin formation in plants is known to be catalyzed enzymatically[111]. It is also known that light accelerates the phaeophytinization[112] and this has been suggested as one explanation of the more pronounced SO_2 effects in the light than in the dark. Chl *a* tends to be destroyed at a faster rate than Chl *b*, as can be seen from *in vivo* and *in vitro* studies.[113, 114] This difference in stability can easily be demonstrated by use of acidic agents as sources of SO_2, which convert Chl *a* to phaeophytin *a*. However, rapid phaeophytin formation has only been observed in studies with very high SO_2 concentrations or together with low pH. These situations rarely exist in the field, and phaeophytin formation may have very little relevance to the decrease of photosynthesis found in SO_2 exposed lichens or higher plants.[67,113,115]

The observed pigment changes differ widely from one species to another, and even between varieties of the same species.[60] There are e.g. different responses within the same algal species in various lichens.[116] However, in the lichen investigation reported here no chlorophyll degradation was observed (I). This is in accordance with several other investigations, where the rate of photosynthesis in short time experiments is affected before any effect on the chlorophyll could be detected.[47,113,48,116,117]

The *in vivo* chlorophyll destruction by SO_2 is complex to evaluate.

In a series of investigations on lichens and on spinach chloroplasts, it has been shown that SO_2 induced Chl bleaching is associated with a spectral absorption shift towards shorter wavelengths.[47,118,116] Light is required for bleaching to occur and the effects resemble those found with other oxidants, e.g. permanganate[116,118]. The mechanism of chlorophyll destruction is not explained, however, the most likely reaction is an oxidation of the pigment molecule.

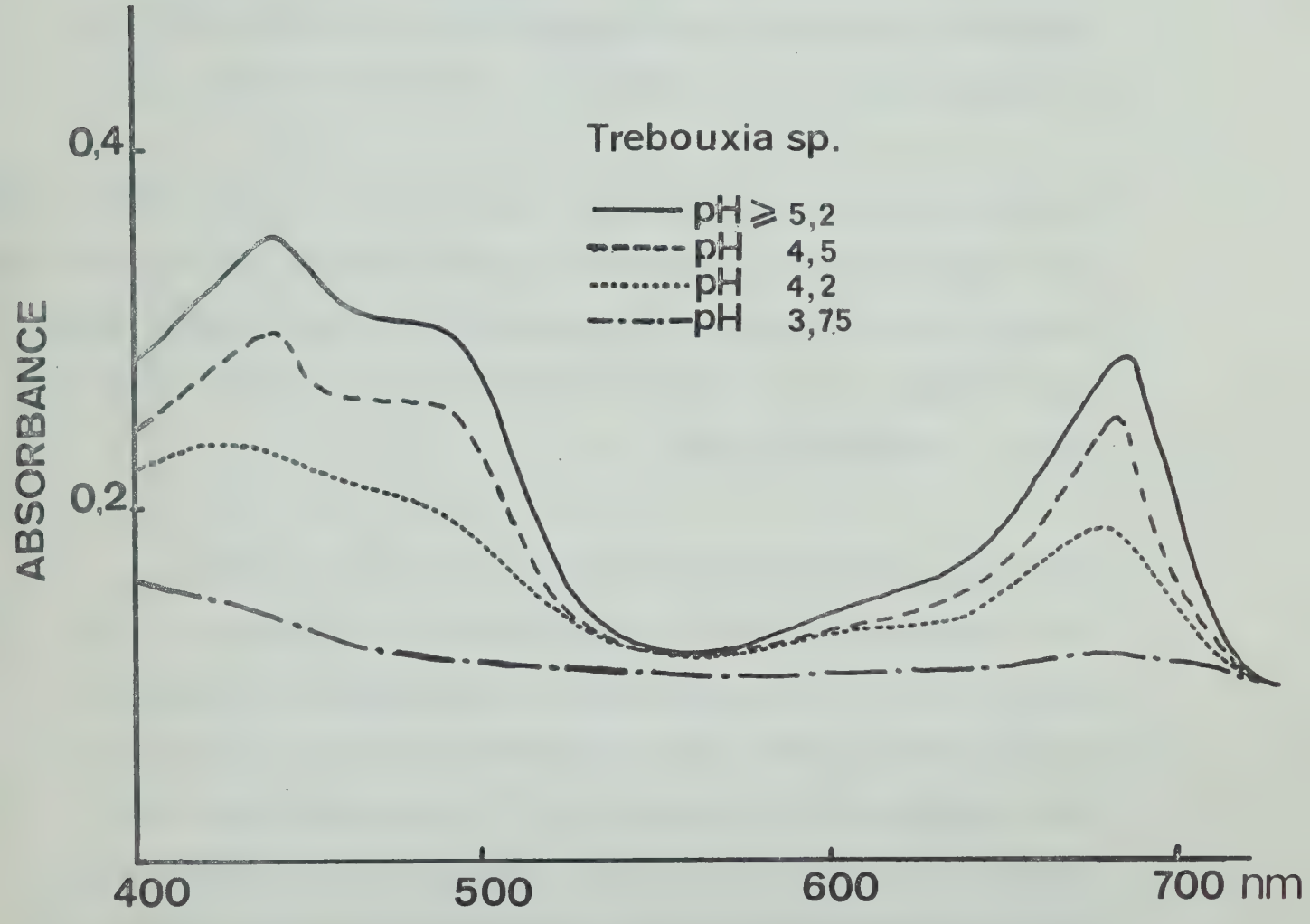


FIGURE LEGEND

Absorption spectra of Treboxia sp. isolated from Hypogymnia physodes L (Ny1) showing the effects of SO_2 (12,5 ppm in solution) after 24 hours of treatment in buffered media at different pH's. The pigment degradation is most rapid at low pH (3,75) while SO_2 at higher pH (5,2 or above) is without effect. The degradation is accompanied with a shift in absorption towards shorter wavelengths in the red part of the spectrum. No pigment degradation was detected at pH 3,75 if SO_2 was omitted. Figure from Sundström and Hällgren Ambio 1973.[119]

It was recently demonstrated in experiments with Pinus contorta that the effect of SO_2 on pigment breakdown was due to a specific effect on chlorophyllase and the observed reaction was not caused by increased acidity.[120] Malhotra reported that 10-50 ppm SO_2 in solution caused a significant increase in chlorophyllase activity and Chl b was converted to the corresponding chlorophyllid b by removal of the phytol group. However, it is possible that this enzyme is associated with a lipoprotein chlorophyll complex and inactive in vivo.[111] Hence, the influence of SO_2 on this hydrolytic reaction remains to be fully investigated.

In the study on Pinus silvestris (V), and with the SO_2 concentrations used (V) no degradation of chlorophyll(s) has been detected. High SO_2 concentrations may cause a lowering of the chlorophyll a/b ratio in pine needles, which indicates that chlorophyll a is more influenced than chlorophyll b when chl degradation occurs.

From in vitro studies at low pH, Peiser and Yang[121] have suggested that free radicals produced by aerobic oxidation of bisulphite in the light are involved in the destruction of chlorophylls. Above pH 5 in the light there was very little chlorophyll destruction. In the dark chlorophyll destruction occurred if Mn^{2+}, O_2 and glycine were added. Chlorophyll destruction was linked to bisulphite oxidation, anf free radical scavengers were effective in inhibiting the destruction of chlorophylls in both systems. Whether the free radicals produced during the aerobic oxidation of bisulphite are responsible for Chl destruction in vivo remains to be investigated.

The Chl content of chloroplasts fluctuates, and these natural changes during a season are due to adaptations in the structure and function of the chloroplast membrane to climatic influence. It can be assumed that during periods of adaptation, e.g. during spring or autumn the chloro-

plast membrane might be more sensitive to attacks by radicals or oxidizing/reducing agents. Reports to what extents these natural variations can be changed(delayed/enhanced)by SO_2 are scarce.

LICHEN EXPOSURE STUDIES

When SO_2 is dissolved in water a number of ionic states in equilibrium can be assumed, and depending upon the pH, SO_2 acts as a reducing or oxidizing agent. SO_2 becomes an increasingly better oxidizing agent as the pH in the medium is lowered [118]. The pH value inside cells and chloroplasts are difficult to measure but optimum pH values for enzymes of the reductive pentose phosphate cycle are around 7-8 in most plants. In this pH range HSO_3^- and SO_3^{2-} will be present, and SO_3^{2-} will be predominant at the higher pH [98]. It must be noted that the effective redox potentials are also dependent on the concentration and nature of other solutes and thermodynamic predictions based on reduction potentials are of course limited if the kinetics of a reaction are unfavorable. Based on penetration arguments it has furthermore been proposed [122] that HSO_3^- and SO_3^{2-} are of minor importance since the nonionic form of dissolved sulphur dioxide ($SO_2 \times \cdot H_2O$) is the principal species crossing cell membranes.

One limitation in all lichen studies with aqueous exposures of SO_3^{2-} or HSO_3^- has been the inability to relate the results to those of gaseous fumigation. Recently Nieboer et al. [123] evaluated a model for the relationship, at 18 °C, which was linear for the gaseous concentrations range 0-5 ppm.

Very little is known about the effects of SO_2 on lichen nitrogen fixation and photosynthesis from field studies. Kallio and Varheenmaa [124] reported that nitrogen fixation was more inhibited than photosynthesis when Stereocaulon paschale and Nephroma articum were transplanted into a city (Åbo) with an unknown mixture of air pollutants.

In the study presented in this thesis (I) $NaHSO_3$ has been used at different pH's, to mimic the SO_2 action. The inhibition of both nitrogen fixation and photosynthesis by HSO_3^-, was much increased at low pH. The pH dependence is in accordance with earlier studies on lighen photosynthesis. The effect of SO_2 in lichen studies has often been attributed to acidifying. The most sensitive lichen species in the field are generally those with the lowest buffering capacity for acid substances[24]. It is evident that the buffering system of any plant may be momentarily overloaded if high concentrations of SO_2 are used. In most cells the major buffering substances operative in the range pH 6-8 are phosphate compounds with a pk_a (6,8-7,0) and certain amino acids both free and in proteins[125]. It can be stated from this study (I), where lichens and algae were treated in buffered media, that lowering the pH, *per se* did not cause effects comparable to those found when HSO_3^- was present. This is also in accordance with earlier findings[48,117,118]. Furthermore the pH change is usually considered to be slow, and e.g. the inhibitory effect on nitrogen fixation was rapid. The inhibition of photosynthesis and nitrogen fixation was light dependent and readily reversible. No good explanation of the observed effects could be given, however, some suggestions were made. In addition, it could have been foreseen, that interference of SO_2 with ATP production or utilization should readily affect the energy requiring fixation of nitrogen.

ECOPHYSIOLOGICAL LICHEN STUDIES

The decline or absence of, especially, epiphytic lichens in cities, industrial or larger areas is a wellknown phenomenon and is often thought to be caused by air pollutants. A number of different methods have been used in lichen/air pollution studies.[22-26] However, the possibility of utilizing physiological reactions e.g. the rate of photosynthesis and nitrogen fixation as a form of rapid biotest in field studies[119] has not been elucidated. The determination of environmental quality might be assisted if one could find a suitable and sensitive lichen species on which one could perform physiological tests in the field. Significant changes (only sometimes specific) in physiological activities may then indicate disturbance at an early stage due to a single pollutant or to an unknown number of pollutants in combination. However, the environment is continously changing, and little is predictable especially in air polluted environments. The complexity of the interactions among variables, and the significance of these environmental fluctuations to lichen thalli, or physiological processes in lichen thalli, is yet only hinted at.

It is only by reference to the field situation that laboratory work on pollutants has any validity, and it is, at best, through laboratory work that the field situation becomes understandable. Much more work is needed on the physiological side, to obtain more information about underlying mechanisms of responses to air pollutants. This is perhaps especiall true for lichens. An exellent review of lichen physiology and related problems is given by Farrar.[126]

It must be understood that research into the lichen versus fluoride (F) pollution problem is still in its infancy. In the studied areas, F de-

serts does not exist, however, the lichen flora is changing but is not yet in equilibrium with the pollution. The pollutant is in form of HF and in particulate form, and the lichens are subjected to constantly changing concentrations of the pollutants.

The choice of parameters used to assess the response of a lichen is also difficult. In order to relate pollution to its source of emission lichens can be transplanted from unpolluted sites to "ecologically similar" but polluted sites. Gas exchange measurements are most commonly used as a diagnostic tool in transplant studies.[127,128] This approach has been used (III) and there is in many cases a good negative correlation between mean relative growth rate and fluoride accumulation. It must be noted that it is impossible to do a satisfactory control transplant, the reciprocal transplant is hardly a control. Photosynthesis, in Cladonia rangiferina, was also negatively correlated to observed fluoride content of the lichen, in contrast respiration was not. The measurements were performed in the laboratory under one set of conditions, which of course limits the possibility of drawing any firm conclusions.

$^{14}CO_2$ incorporation of Parmelia olivacea (III) or Hypogymnia physodes (II) has been measured in samples collected from different areas around sintering plants. A standardized sampling has been used which minimized site differences. In addition, only marginal parts, which are metabolically more active than central and older parts of the thalli, have been used. This also helps to reduce the variability, and simplify comparisons. Under these conditions it is evident that lichen photosynthesis is reduced in areas subjected to HF, SO_2 and other pollutants in air in combination (II, III). The correlation with the observed HF content in the lichen Parmelia olivacea was, however, not directly obvious, with the exception of the area close to the emission source. The reason for this is not known. Nitrogen fixation in Nephroma articum is also reduced near

the emission source, as shown in II.

It is evident from the study (III) that there are considerable differences between lichen species in the response to F. Alectoria simplicior is the most sensitive species observed in the area. By measuring the length of thalli, it is indicated that part of the population is removed by selection.

It can be concluded that combining field and laboratory investigations of effects of air pollutants must be a central, challenge in the future.

CONSIDERATIONS OF MEASUREMENTS OF SO_2 EFFECTS ON PHOTOSYNTHESIS OF PINE TREE SEEDLINGS

In order to separate the response of plants to their physical environment from physiological changes induced by air pollutants, such as SO_2, an appropriate quantitative analysis has to be used.[129] This has partly been foreseen in several of the earlier more descriptive investigations. More rencently, however, the interest has been focused on realistic levels of pollutant concentrations, affecting photosynthesis and growth of pine trees.[130] The measurement of photosynthesis can be performed in several ways and on different levels of organisation. The construction of the apparatus for measuring quantum yield and quanta absorption spectra of intact plants, can be regarded as one approach for linking gas exchange research on intact plants with research at the cellular and biochemical level (IV). By measuring the quantum flux in the sphere, together with the CO_2-flux, the quantum yield of photosynthesis of intact plants can be determined. In addition the spectral distribution of the absorbed photon flux density and the absorbance spectrum, mainly dependent upon the concentrations of leaf pigments and leaf structure, can be defined. Hereby the apparatus should make it possible to study effects of environmental factors affecting photosynthesis adequately.

It is evident that high concentrations of SO_2 rapidly inhibit the net photosynthetic rate of primary needles of pine trees (V). The inhibition is readily reversible. An effect of the CO_2 flux by 'medium' SO_2 concentrations is observed at high photon flux densities absorbed by the plant. An inhibition of the Calvin cycle might be suggested. The result reported here shows that a decrease of the amount of RuBP-carboxylase in the seedlings follows fumigation with SO_2.[101,106,108] In contrast to earlier findings, a noncompetitive inhibition of RuBP-carboxylase by SO_3^{2-} with respect to HCO_3^-

is indicated *in vitro*. However, this inhibition found *in vitro* need not be the mechanism responsible for the observed effect *in vivo*. From the results reported here, an effect on photophosphorylation or ATP-utilization can not be excluded. The quantum yield of photosynthesis is not affected as long as the CO_2-exchange is limited solely by the absorbed quantum flux. Hence an effect on electron transport can not be excluded from these measurements.

CONCLUSION

In spite of its long history as an air pollutant, SO_2 has received fairly little attention from plant physiologist and biochemists.

The reactions of the plant system to SO_2- the responses - are ultimately molecular in basis, but need to be regarded at several different levels of organisation in the plant. There exists a discrepancy between mechanism studies on isolated organelles, membrane systems or enzymes and the effect studies on intact plants. To clarify the action of SO_2 on photosynthesis, the fundamental processes of photosynthetic electron transport, photophosphorylation and CO_2 reduction should be investigated in parallel with studies of the absorbed quantum flux and the accompanying processes of CO_2 flux and SO_2 flux between air and leaf. Accompanied by physiological studies, those biochemical mechanisms which are immedieately affected by air pollutants might be identified. Another central challenge is to connect laboratory and field studies.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to all the members of the Department of Plant Physiology, University of Umeå, who have contributed with both stimulating discussion and technical assistance. I wish to express my gratitudes to my superadvisor Docent Karl-Ragnar Sundström for his support and encouragement during the course of this work. Thanks are also due to Professor Lennart Eliasson for his interest and valuable advice. For fruitful collaboration, I thank FK Laila Brunes, FK Per-Anders Esseen, Docent Kerstin Gezelius, FK Kerstin Huss-Danell, Docent Bengt Nyman, FK Göran Sandberg and Docent Gunnar Öquist. This work could not have been accomplished without the valuable advice, encouragement and never failing spirit of Docent Gunnar Öquist and the other members of the photosynthetic group at this Department. I am indepted to Mrs. Inga-Lill Nilsson and Miss Agneta Ehman for patiently converting my usually illegible manuscript into presentable form.

This work has been supported by grants from the National Swedish Environment Protection Board.

REFERENCES

1. Katz, M. 1949. Ind. Eng. Chem. 41. 2450-2465.
2. Thomas, M.D. 1951. Ann. Rev. Plant. Physiol. 2. 293-322.
3. Thomas, M.D. 1961. WHO. Monograph Ser. 46. 233-278. Geneva.
4. Webster, C.C. 1967. Agr. Res. Counc. London.
5. Garber, K. 1967. Luftverunreinigung und ihre Wirkungen 1-279. Gebrüder Borntraeger Berlin-Nikolassee.
6. Brandt, C.S. and Heck, W.W. 1968 in Air Pollution (Stern, A.C. ed.) vol. 1. 401-443. Chap. 14. Academic Press, New York.
7. Berge, H. 1970. Handbuch der Pflanzenkrankheiter. 1(4) 1-169. P. Parey Berlin und Hamburg.
8. Linzon, S.N. (ed.) 1970. Impact of Air Pollution on Vegetation. A.P.C.A. Pittsburg, Penn.
9. Treshow, M. 1970. (ed.) Environment and Plant Response. McGraw Hill. New York - London - Toronto.
10. Tamm, C.O. and Aronsson, A. 1972. Research Notes No. 12. Royal College of Forestry, Stockholm.
11. Jensen, K.F., Dochinger, L.S., Roberts, B.R. and Townsend, A.M. 1976. in Proceedings in Life Sciences. Modern Methods in Forest Genetics. 189-216 (Miksche, J.P. ed). Springer Verlag, Berlin, Heidelberg, New York.
12. Knabe, W. 1976. Ambio 5-6.
13. Haut, van, H. and Stratman, H. 1970. Farbtafelatlas über Schwefeldioxid-Wirkungen an Pflanzen, Verlag W Girardet Essen.
14. Jacobson, J.S. and Hill, A.C. 1970. A.P.C.A. Pittsburg, Pennsylvania.
15. Hindawi, I.J. 1970. Air Pollution Injury to Vegetation, U.S. Dept. HEW, AP-71.

16. McCune, D.C. 1973. Adv. Chem. Ser. 122. 48-62.

17. Ziegler, I. 1973. in Env. Qual. Safety. (Coulston, F. ed.) vol. II, 182-208. Stuttgart.

18. Ziegler, I. 1975. Res. Review. 79-104.

19. Mudd, J.B. 1975. in Responses of Plants to Air Pollution. (Mudd, J.B. and Kozlowski, T.T. eds.). Academic Press, New York, San Francisco, London.

20. Malhotra, S.S. and Hocking, D. 1976. New Phytol. 76. 227-237.

21. Horsman, D.C. and Wellburn, A.R. 1976 in Effects of air pollutants on plants (Mansfield, T.A. ed.) 192-196.

22. Ferry, B.W., Baddeley, M.S., Hawksworth, D.L. 1973. Air pollution and lichens, The athlone Press. London.

23. Gilbert, O.L. 1973. in the lichens (Ahmadjian, V. and Hale, M.S. eds.). Academic Press, New York and London. Ch. 13. 443-469.

24. Le Blanc, F. and Rao, D.N. 1975 in Responses of Plants of Air Pollution (Mudd, J.B. and Kozlowski, T.T. eds.). 237-272.

25. Hawksworth, D.L. 1976. Nautral History Book reviews. 8-15.

26. Gilbert, O.L. 1976 in Proceedings of the Kuopio meeting on plant damages caused by air pollution (Kärenlampi, L. ed.).

27. Dässler, H.G. 1976. Einfluss von Luftverunreinigungen auf die Vegetation. Veb Gustav Ficher Verlag. Jena.

28. Guderian, R. 1977. Air Pollution. Springer-Verlag, Berlin, Heidelberg, New York.

29. Willix, R. 1976. in Effects of air pollutants on plants (Mansfield, T.A. ed.). 161-184.

30. Bull, J.N. and Mansfield, T.A. 1974. Nature vol 250. pp. 443-44.

31. White, K.L., Hill, A.C. and Bennett. 1974. Env. Sci. & Technol. Vol 8 nr 6, 574-76.

32. Thomas, W.A., Goldstein, G., Wilcox, W.H. 1973. Biological Indicators of Environmental Quality. Ann Arbor Science publ. inc. Mich.

33. De Wit, T. 1976. Epiphytic lichens and air pollution in the Netherlands. Bibliotheca Lichenologia 5.

34. Houston, D.B. 1974. Can. J. For. Res. 4: 65-68.

35. Börtitz, S. 1964. Biol. Zbl. 83: 501-513.

36. Schönbach, H., Dässler, H.G., Enderlein, H., Bellman, E. and Kästner, W. 1964. Züchter 34(8): 312-316.

37. Dochinger, L.S., Bender, F.W., Fox, F.O. and Heck, W.W. 1970. Nature 225:476

38. Hodges, G.H., Menser, H.A., Jr. and Ogden, W.B. 1971. Agron. J. 63: 107-111.

39. Menser, H.A., Hodges, H.A. and McKee, G.G. 1973. J. Environ. Qual. 2: 253-258.

40. Taylor, G.E. Jr., and Murdy, W.A. 1975. Bot. Gaz. 136 (2): 212-215.

41. Bell, J.N.B. and Mudd, C.H. 1976. In Effects of air pollutants on plants (Mansfield, T.A. ed.), 87-103.

42. van Haut, H. 1961. Staub 21(2): 52-56.

43. Cracker, L.E. and Starbruck, Y.S. 1973. Environ. Res. 6: 91.

44. Krasnowskij, A.A. 1969. Prog. Photosyn. Res. 2: 709.

45. Libera, W., Ziegler, H. and Ziegler, I. 1973. Planta 9:269.

46. Ziegler, I. and Libera, W. 1975. Z. Naturforsch. 30 c. 634-37.

47. Puckett, K.J., Richardson, D.H.S., Flora, W.P. and Nieboer, E. 1974. New Phytol. 73: 1183-1192.

48. Hill, D.J. 1974. New Phytol. 73: 1193-1205.

49. Mansfield, T.A. 1976. in Commentaires in Plant Science (Smith, H. ed.) 13-22. Pergamon Press.

50. Pahlich, E. 1975. Atmos. Environm. vol. 9. 261-63.

51. Nielsen, J.P. 1968. cited from Daines, R.H. 1968. J. occup. med. vol. 10:9. 516-523.

52. Schiff, J.A. and Hodson, R.C. 1973. Annu. Rev. Plant, Phys. 24; 381-414.

53. MacLeod, R.M., Farkas, W., Fridovitch, I., Handler P.J. 1961. J. Biol. Chem. 236; 1841.

54. Asada, K. and Kiso, K. 1973. Eur. J. Biochem. 33; 253-257.

55. McCord, J.M. and Fridovitch, I. 1969. J. Biol. Chem. 244; 6056.

56. Schwenn, J.D., Depka, B. and Hennies, H.H. 1976. Plant Cell Physiol. 17: 165-176.

57. Trebst, A. and Schmidt, A. 1969. in Progress in photosynthesis research (Metzner, H. ed.) vol. 3. 1510.

58. Schmidt, A. and Trebst, A. 1969. Biochim. Biophys. Acta. 180; 529.

59. Hennies, H. 1975. Z. Naturforsch. 30 c. 359-62.

60. Roy, A.B. and Trudinger, P.A. eds. 1970. The Biochemistry of Inorganic Compounds of Sulphur. University Press.

61. Asahi, T. 1964. Biochim. Biophys. Acta. 82; 58-66.

62. Brunold, Chr. and Erismann, K.H. 1975. Experientia 3213.

63. de Cormis, L. 1969. in Air Pollution Proc. 1st Eur. Congr. Influence of Air Pollution on Plants and Animals. Wageningen. 75-78.

64. Fankhauser, H., Brunold, Chr. and Erismann, K.H. 1976. Oecologia 23: 201-209.

65. Brinkman, E., Lüttge. U. and Fischer, K. 1971. Ber. Dtsch. Bot. Ges. Bd. 84(9): 523-524.

66. Lüttge, U., Osmond, C.B., Ball, E., Brinckmann, E. and Kinze, G. 1972. Plant Cell Physiol. 13; 505.

67. Puckett, K.J., Tomassini, F.D., Nieboer, E. and Richardson, D.H.S. 1977. New Phytol. 79. 135-145.

68. Wellburn, A.R., Majernik, O. and Wellburn, A.M. 1972. Environm. Pollut. 76; 83-96.

69. Fischer, K., Kramer, D. and Ziegler, H. 1973. Protoplasma 76: 83-96.

70. Godzik, S. and Sassen, M.M.A. 1974. Phytopath. Z. 79: 155-159.

71. Thomson, W.W. 1975. In: Responses of Plants to Air Pollution (Mudd, J.B. and Kozlowski, T.T., eds.). Academic Press Inc. 179-194.

72. Ikonen, S. and Kärenlampi, L. 1976. In: Proc. Kuopio Meet. Pl. Dam. Caus. Air Poll. (Kärenlampi, L., ed.), Kuopio. 37-45.

73. Malhotra, S.S. 1976. New Phytol. 76: 239.

74. Cecil, R. and Wake, R.G. 1962. Biochem. J. 82; 401.

75. Kahn, A.A. and Malhotra, S.S. 1977. Phytochemistry vol. 16. 539-543.

76. Asada, K., Kitoh, S., Deura, R. and Kazai, Z. 1965. Pl. Cell Physiol. 6: 615.

77. - , Deura, R. and Kasai, Z. 1968. Pl. Cell Physiol. 9; 143-146.

78. Hall, D.O. and Telfer, A. 1969. Prog. Photosynth. Res. 3: 1281-87.

79. Silvius, J.E., Ingle, M. and Baer, C.H. 1975. Pl. Physiol. 56: 434-437.

80. Baldry, C.W., Cockburn, W. and Walker, D.A. 1968. BBA 153: 476-483.

81. Plesničar, M. 1977. 4th Int. Congr. on Photosynt. Reading UK. 299.

82. Pick, U. and Avron, M. 1973. BBA 325: 297-303.

83. Ballantyne, D.J. 1973. Phytochem. 12: 1207-9.

84. Gromet-Elhanan, Z. and Leiser, M. 1973. Arch. Biochem. Biophys. 159: 583-589.

85. Roy, H. and Moudrianakis, E.N. 1971. Proc. Natl. Acta. Sci. U.S. 68: 2720-2724.

86. Datta, D.B., Ryrie, I.J. and Jagendorf, A.T. 1974. J. Biol. Chem. 249: 4404.

87. Ryrie, I. and Jagendorf, A.T. 1971. J. Biol. Chem. 246; 582-588.

88. Grebanier, A.E. and Jagendorf, A.T. 1977. BBA 459; 1-9.

89. Jagendorf, A.T. 1975. In: Bioenergetics of Photosynthesis (Govindjee, ed.). Academic Press, 414-492.

90. Pfleiderer, G., Jeckel, D. and Wieland, T. 1956. Biochem. Z. 328; 187.

91. Shih, N.T. and Petering, D.H. 1973. Biochem. Biophys. Res. Commun. 55: 1319.

92. Petering, D.H. and Shih, N.T. 1975. Environm. Res. 9: 55-65.

93. Williams, R.J.P. 1973. Biochem. Society Transactions. 1: 1-26.

94. Haywood, G.C., Hill, H.A.O., Pratt, J.M., Vanston, N.J. and Williams, R.J.P 1965. J. Chem. Soc. 6: 485-493.

95. Fischer, K. 1971. Mitt. Forstl. Vers. Anst. Wien. 92: 209-23.

96. Haisman, D.R. 1974. J. Sci. Fd Agric. 25: 803-810.

97. Jäger, H.J. and Pahlich, E. 1976. Int. J. Environm. Anal. Chem. 4(4): 257-262.

98. Schroeter, L.C. 1966. "Sulfur Dioxide. Applications in Foods, Beverages and Pharmaceuticals". Pergamon Oxford.

99. Tanaka, H., Takanashi, T. and Yatazawa, M. 1972. Water, Air and Soil Pollution 1: 205-211.

100. Jiráček, V., Machácková, I. and Kostír, J. 1972. Experientia Vol 28(9): 1007-1008.

101. Ziegler, I. 1972. Planta 103: 155-163.

102. Ziegler, I. 1973. Phytochemistry 12: 1027.

103. Ziegler, I. 1974. B.B.A. 364: 28.

104. Ziegler, I. 1974. Phytochemistry 13: 2403.

105. Zielger, I., Marewa, A., Schoepe, E. 1976. Phytochemistry 15: 1627-1632.

106. Ziegler, I. 1977. Oecologia 28, 147-151.

107. Pierre, M. 1977. Physiol. Vég. 15(1), 195-205.

108. Martinovic, B. and Plesnicar, M. 1977. 4th Int. Congr. on Photosyn. Reading 240.

109. Anderson, L.E. and Avron, M. 1976. Plant Physiol. 57: 209-213.

110. Anderson, L.E. and Duggan, J.X. 1977. Oecologia 28, 147-151.

111. Holden, M. 1961. Biochem. J. 78: 359.

112. Ferenbaugh, R.W. and Bilderback, D.E. 1974. Am. J. Bot. 61 (suppl).

113. Richardson, D.H.S. and Puckett, K.J. in Air Pollution and Lichens (Ferry, B.W. et al. eds.) the Athlone Press. ch 13. 283-298.

114. Katz, M. and Shore, V.C. 1955. J. Air Poll. Contrl. Ass. Vol 5(3): 144-150, 182.

115. Jensen, K.F. and Kozlowski, T.T. 1974. Proc. 3rd North. Am. For. Biol. Workshop, p. 359.

116. Nieboer, E., Richardson, D.H.S., Puckett, K.J. and Tomassini, F.D. 1976. in Effects of Air Pollutants on Plants (Mansfield, T.A. ed.). Cambridge University Press. 61-85).

117. Showman, R.E. 1972. Bryologist. 75: 335-341.

118. Puckett, K.J., Nieboer, E., Flora, W. and Richardson, D.H.S. 1973. New Phytol. 72: 141-54.

119. Sundström, K.-R. and Hällgren, J.-E. 1973. Ambio 2: 13-21.

120. Malhotra, S.S. 1977. New Phytol. 78: 101-109.

121. Peiser, G.D. and Yang, S.F. 1977. Plant Physiol. 60: 277-281.

122. Hocking, D. and Hocking, M.B. 1977. Environ. Pollut.

123. Nieboer, E., Tomassini, F.D., Puckett, K.J. and Richardson, D.H.S. 1977. New Phytol. 79: 157-62.

124. Kallio, S. and Varheenmaa, T. 1974. Rep. Kevo Subartic Res. Stn. 11: 42-46.

125. Raven, J.A. and Smith, F.A. 1976. Current Advances in Plant Science. No. 24. vol. 8. No 5.

126. Farrar, J.F. 1973. in op. cit. 22. 238-282.

127. Kauppi, M. 1976. Flora. Bd. 165. 407-414.

128. Ikonen, S. and Kärenlampi, L. 1976. in Proceedings of the Kuopio meeting on plant damages caused by air pollution (Kärenlampi, L. ed.) Kuopio. 37-45.

129. Unsworth, M.H., Biscoe, P.V. and Black, V. 1976. in Effects of air pollutants on Plants (Mansfield, T.A. ed.). Cambridge University Press. 5-16.

130. Farrar, J.F., Relton, J. and Rutter, A.J. 1977. J. appl. Ecol. 14: 861-875.